IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Denney
Serial No.:
Filed: 08/09/01
Entitled: **VACCINES FOR TREATMENT OF LYMPHOMA AND LEUKEMIA**

Group No.: Not Assigned
Examiner: Not Assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.10

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Dated: August 9, 2001

By: Susan M. McClintock
Susan M. McClintock

Sir or Madam:

Prior to the examination of this Application, Applicant respectfully requests that the following amendments be entered.

IN THE SPECIFICATION:

On page 1, line 1, please delete the current title "Vaccines for Treatment of Lymphoma and Leukemia", and insert --Gene Amplification Methods-- as the title of the invention.

On page 1, between lines 1 and 2, please insert --The present Application is a Continuation of pending U.S. Patent Application Serial No. 09/370,453, filed August 9, 1999, which is a Divisional of U.S. Patent Application Serial No. 08/761,277, filed December 6, 1996, now U.S. Patent 5,972,334, which is a Continuation-in-part of U.S. Patent Application Serial No. 08/644,664, filed May 1, 1996, now U.S. Patent 5,776,746.--.

IN THE CLAIMS:

Please cancel Claims: 1-24.

Please add the following new claims:

25. (new) A method of co-amplifying recombinant oligonucleotides, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) introducing said expression vector and said amplification vector into said parent cell line to generate transformed cells, wherein a ratio ranging from 2:20 to 2:50 of said amplification vector to said expression vector is employed;

c) introducing said transformed cells into a first aqueous solution containing an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

26. (new) The method of Claim 25, wherein said expression vector and said amplification vector are linearized prior to introduction into said parent cell line.

27. (new) The method of Claim 25, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

28. (new) The method of Claim 25, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

29. (new) The method of Claim 25, further comprising the steps of:

i) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

ii) identifying at least one transformed cell capable of growth in said second aqueous solution.

30. (new) A method of co-amplifying recombinant oligonucleotides, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a selection vector comprising a third recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

iv) a T lymphoid parent cell line;

b) introducing said expression vector, said amplification vector and said selection vector into said cell line to generate transformed cells, wherein a ratio

ranging from 1:2:20 to 1:2:50 of said selection vector to said amplification vector to said expression vector is employed;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

d) identifying at least one transformed cell capable of growth in said first aqueous solution;

e) introducing said transformed cell capable of growth into said first aqueous solution in a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

31. (new) The method of Claim 30, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

32. (new) The method of Claim 30, wherein said selection vector encodes an active hypoxanthine guanine phosphoribosyltransferase.

33. (new) The method of Claim 30, wherein said expression, amplification and selection vectors are linearized prior to introduction into said T lymphoid parent cell line.

34. (new) The method of Claim 30, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

35. (new) A method of producing a vaccine for treatment of B-cell lymphoma, comprising:

a) providing:

i) malignant cells isolated from a patient having a B-cell lymphoma;

ii) an amplification vector comprising a recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a T lymphoid parent cell line;

b) isolating from said malignant cells nucleotide sequences encoding at least one V_H region and at least one V_L region, said V_H and V_L regions derived from immunoglobulin molecules expressed by said malignant cells;

c) inserting said nucleotide sequence encoding said V_H region into a first expression vector, and inserting said nucleotide sequence encoding said V_L region into a second expression vector;

d) introducing said first and second expression vectors and said amplification vector into said parent cell to generate transformed cells, wherein a ratio ranging from 2:20 to 2:50 of said amplification vector to said first or second expression vector is employed;

e) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth expresses said V_H and V_L regions.

36. (new) The method of Claim 35, wherein said nucleotide sequences encoding said V_H and V_L regions comprise at least two V_H and at least two V_L regions.

37. (new) The method of Claim 35, wherein at least one of said expression vectors is linearized prior to introduction into said parent cell line.

38. (new) The method of Claim 35, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

39. (new) The method of Claim 35, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

REMARKS

Claims 1-24 were filed in the accompanying Continuation Application. The above amendment cancels Claims 1-24, and adds new Claims 25-39. As such, Claims 25-39 are currently pending in this Application.

Dated: August 9, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In The Specification:

Title beginning at line 1, page 1, has been amended as follows:

[Vaccines for Treatment of Lymphoma and Leukemia] Gene Amplification Methods

On page 1, between lines 1 and 2, the following text is inserted:

The present Application is a Continuation of pending U.S. Patent Application Serial No. 09/370,453, filed August 9, 1999, which is a Divisional of U.S. Patent Application Serial No. 08/761,277, filed December 6, 1996, now U.S. Patent 5,972,334, which is a Continuation-in-part of U.S. Patent Application Serial No. 08/644,664, filed May 1, 1996, now U.S. Patent 5,776,746.

In the Claims:

Claims 1-24 have been cancelled.

Claims 25-39 has been added.

COMPLETE SET OF PENDING CLAIMS

25. A method of co-amplifying recombinant oligonucleotides, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) introducing said expression vector and said amplification vector into said parent cell line to generate transformed cells, wherein a ratio ranging from 2:20 to 2:50 of said amplification vector to said expression vector is employed;

c) introducing said transformed cells into a first aqueous solution containing an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

26. The method of Claim 25, wherein said expression vector and said amplification vector are linearized prior to introduction into said parent cell line.

27. The method of Claim 25, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

28. The method of Claim 25, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

29. The method of Claim 25, further comprising the steps of:

i) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

ii) identifying at least one transformed cell capable of growth in said second aqueous solution.

30. A method of co-amplifying recombinant oligonucleotides, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a selection vector comprising a third recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

iv) a T lymphoid parent cell line;

b) introducing said expression vector, said amplification vector and said selection vector into said cell line to generate transformed cells, wherein a ratio ranging from 1:2:20 to 1:2:50 of said selection vector to said amplification vector to said expression vector is employed;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

d) identifying at least one transformed cell capable of growth in said first aqueous solution;

e) introducing said transformed cell capable of growth into said first aqueous solution in a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

31. The method of Claim 30, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

32. The method of Claim 30, wherein said selection vector encodes an active hypoxanthine guanine phosphoribosyltransferase.

33. The method of Claim 30, wherein said expression, amplification and selection vectors are linearized prior to introduction into said T lymphoid parent cell line.

34. The method of Claim 30, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

35. A method of producing a vaccine for treatment of B-cell lymphoma, comprising:

a) providing:

i) malignant cells isolated from a patient having a B-cell lymphoma;

ii) an amplification vector comprising a recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a T lymphoid parent cell line;

b) isolating from said malignant cells nucleotide sequences encoding at least one V_H region and at least one V_L region, said V_H and V_L regions derived from immunoglobulin molecules expressed by said malignant cells;

c) inserting said nucleotide sequence encoding said V_H region into a first expression vector, and inserting said nucleotide sequence encoding said V_L region into a second expression vector;

d) introducing said first and second expression vectors and said amplification vector into said parent cell to generate transformed cells, wherein a ratio ranging from 2:20 to 2:50 of said amplification vector to said first or second expression vector is employed;

e) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth expresses said V_H and V_L regions.

36. The method of Claim 35, wherein said nucleotide sequences encoding said V_H and V_L regions comprise at least two V_H and at least two V_L regions.

37. The method of Claim 35, wherein at least one of said expression vectors is linearized prior to introduction into said parent cell line.

38. The method of Claim 35, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said T lymphoid parent cell line.

39. The method of Claim 35, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.